Minimal Residual Disease (MRD) as a Surrogate Endpoint in Acute Lymphoblastic Leukemia (ALL) Workshop

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Workshop Objectives

1. Qualification of early MRD as an efficacy/response biomarker
2. Consensus position on early MRD (critical threshold value) as a surrogate endpoint
3. Consensus on preferred technology platform for MRD determination with defined performance characteristics
4. Consensus on standardized methodology and need for centralized performance
5. Determine need for FDA approved In Vitro Diagnostics (IVD)

Minimal Residual Disease (MRD) in Acute Lymphoblastic Leukemia (ALL): Qualification of an Important Prognostic Indicator as an Efficacy/Response Biomarker and Surrogate Endpoint for Clinical Trials of New Therapeutic Agents

Multiple reports now suggest that detection of minimal residual disease (MRD) at an early time point (during/following Induction or Consolidation therapy) has emerged as a powerful and independent predictor of prolonged event free survival (EFS) in children and adults with acute lymphoblastic leukemia (ALL) (Reviewed in Campana, 2010 and Cazzaniga et al., 2011). In most cases of ALL treated with current, state of the science, multi-agent chemotherapy regimens, early MRD has surpassed, in importance, other widely used and accepted prognostic indicators. As a prognostic biomarker, assessment of MRD has had a profound impact world-wide on the design and conduct of clinical trials in ALL for all known risk groups of patients, currently defined by longstanding, accepted clinical and biological prognostic factors. As standardized methodologies for MRD detection have improved, the evaluation of MRD as an *in vivo* marker of early therapeutic efficacy has allowed for the allocation of patients to different risk-based treatments in an attempt to both improve outcome and mitigate the risk of short and long term toxicity of therapy. Early response to treatment is increasingly being measured by assessing the number of residual ALL cells by their clonal rearrangement of immunoglobulin(Ig) and T cell receptor (TcR) genes, fusion gene expression, and/or leukemia associated immunophenotype (LAIP). Sensitive methods (1 leukemia cell in 10,000 to 100,000 normal cells in a clinical sample) for detection of MRD using assays based on the real time, quantitative polymerase chain reaction (RQ-PCR) and/or flow cytometry (FCM) are being used to risk stratify patients on therapeutic trials designed to adapt treatment for individual patients relative to their risk for treatment failure (Campana, 2009). The decisions for selection of one methodology over another are complex and dependent upon a number of factors.

Methodologies for MRD detection

Reliable and reproducible techniques for the assessment of MRD must exhibit certain performance characteristics. The first of these is sensitivity of at least 10-4 (1 malignant cell in 10,000 normal cells). The specific target value of sensitivity may be highly dependent upon the clinical indication under evaluation or hypothesis being tested. Assay specificity is required to assure discrimination between normal and leukemic cells to avoid false positives. Techniques must be quantifiable within a sizable dynamic range and must demonstrate stability of leukemia-specific markers over time to prevent false negative results especially in long term studies. Given the multi-center nature of large scale clinical trials in ALL, methods and conditions for techniques must be standardized and subject to strict quality control and be available for real time result reporting for utility in clinical decision making (Cazzaniga et al., 2003, 2011; van der Velden et al., 2003, 2007; Campana 2008; Flohr et al., 2008). Both flow cytometric profiling of LAIP and RQ-PCR amplification of fusion transcripts and Ig and TcR genes are widely used for the detection and monitoring of MRD in clinical trials and appear to exhibit all of the necessary prerequisites (Bruggemann et al., 2010).

Somatic chromosomal aberrations in leukemic lymphoblasts are tumor specific and stable and lend themselves for use as PCR targets for MRD detection. As these fusion gene products exist in a minority of ALL cases, the approach is limited and associated with other potential problems including degradation of RNA and the requirement for parallel assessment of a normal housekeeping gene. The application of RQ-PCR of fusion gene transcripts is largely limited to the BCR-ABL1 fusion product in Ph+ ALL. Utilizing this methodology has resulted in the demonstration that MRD levels at end of Induction and the completion of Consolidation are important and highly significant predictors of long term outcome (Pane et al., 2005; Wassman et al., 2005). A more recent study incorporating imatinib with chemotherapy, however, failed to show an association between early MRD determination and long term outcome. Nonetheless, the importance of MRD in making subsequent treatment decisions was noted in the markedly improved EFS in MRD+ patients who underwent HSCT when compared to those who were not transplanted. This supports the premise of the in vivo therapeutic efficacy determination afforded by MRD assessment in Ph+ ALL. Thus, determination of MRD by RQ-PCR of BCR-ABL1 fusion transcripts in Ph+ ALL represents a useful indicator of treatment response and can prove informative to MRD-focused clinical trials in Ph+ ALL. The predictive ability of early MRD assessment in determining long term EFS in children with Ph+ ALL treated with intensive combinations of conventional chemotherapy and BCR-ABL1 directed tyrosine kinase inhibitors is currently under investigation.

Ig and TcR gene rearrangements are extensively utilized as universal MRD targets detected using molecular approaches. Allele-specific oligonucleotides are designed complementary to the specific junctional region target of each patient's leukemic blasts. The technique has been widely used in European studies and the rate of suitable gene rearrangements identified for marker studies approximates 90%. (Campana, 2010; Flohr et al., 2008; Stow et al., 2010; Bruggemann et al., 2006). Since antigen receptor genes may undergo continuing rearrangements as part of clonal evolution, subclones with distinct sequences may be undetected at diagnosis, but become predominant later. Although there have been recommendations to monitor 2 or more different rearrangements (van der Velden et al., 2007), this would be prohibitively stringent and substantially reduce the number of patients for whom MRD detection could be accomplished utilizing this methodology. Only 71% of cases in the AIEOP-BFM ALL 2000 study demonstrated two sensitive targets (Flohr et al., 2008) and productive MRD studies can in fact be performed in cases where there is only one suitable gene rearrangement. To address the concern of false negative results as a result of undetected subclones, the use of another technology, either FCM or RQ-PCR for gene fusion transcripts can be used in tandem. An extensive program in standardization and quality control of RQ-PCR analysis and the development of guidelines for data analysis and interpretation has been accomplished within the "Euro-MRD" group, previously known as the European Study Group for MRD detection in ALL (van der Velden et al., 2007).

LAIPs can be assessed and quantified using multicolor flow cytometry allowing for the detection of at least 4 markers; current techniques and instrumentation allow for eight or more markers. This enhanced capability facilitates discrimination between normal and malignant cell populations, thereby enhancing the sensitivity in MRD determinations as well as permitting the assessment of additional antigen expression representing molecules related to proliferation, cell death, signaling, and drug resistance (Coustan-Smith et al., 2008; Bruggemann et al., 2010).

Ideally, LAIPs should be identified at diagnosis by comparison of cell marker profiles of clinical samples with normal reference samples. However, the use of large panels of antibody combinations permits the detection of critical MRD levels, whereas negative results may be difficult to interpret with certainty.

As well, the potentially confounding effect of transient fluctuations in profiles which may occur as a result of treatment can be minimized by using multiple sets of markers in each case. An accepted level of sensitivity of 0.01% can be achieved in over 90% of patient samples as has been demonstrated in several large studies (Borowitz et al., 2008; Pui et al., 2009). When MRD is present at a level of 0.01% or greater, strikingly similar results are obtained with both FCM and RQ-PCR especially during later time points during therapy (Gaipa et al., 2008, Neale et al., 2004; Irving et al., 2009). This degree of concordance suggests that the techniques are complementary, each with specific strengths and potential limitations (as summarized in Campana, 2010 and Cazzaniga et al., 2011) suggesting that optimal prediction of outcome and risk stratification strategies based on MRD determination can be accomplished with one or both techniques.

Clinical Significance

Early response to therapy has been one of the most important prognostic variables in ALL in children. As a predictor of outcome, early response encompasses host factors, biology of the disease and the specifics of treatment. Early response can be assessed by the reduction/absence of leukemic cells in the bone marrow (measured morphologically) at early time points during multi-agent induction therapy (Gaynon et al., 1990), by the clearance of leukemic blasts in the peripheral blood with a one week prednisone pro-phase (Riehm et al., 1990) and more recently using sensitive techniques to detect MRD. More sensitive measures of MRD including FCM and PCR techniques to identify Ig/TcR rearrangements or fusion transcripts have provided unequivocal evidence of the prognostic importance of MRD levels determined at an early time point in therapy (Cave et al., 1998; van Dongen et al., 1998; Biondi et al., 2000; Coustan -Smith et al., 2000; Panzer-Grumayer et al., 2000; Schmieglow et al., 2001; Borowitz et al., 2008; Conter et al., 2010). In the largest prospective evaluation of more than 3000 patients enrolled in the AIEOP-BFM 2000 study (Conter et al., 2010), MRD detected by standardized and centralized RQ-PCR analysis at two specific early time points, day 33 and day 78, was highly predictive of relapse, replacing nearly all of the conventional clinical and biological factors currently used. Only the known adverse cytogenetic features, hypodiploidy, t(4;11), and t(9;22) remained independently poor prognostic factors. Absence of MRD by RQ-PCR at the end of Induction was the strongest predictor for an excellent outcome (5 yr. EFS >90%). The investigation of early (day 15) MRD by FCM in a series of more than 800 patients by the AEIOP group suggest that early MRD clearance identifies the group of patients with an excellent (5 yr. EFS >95%) prognosis (Basso et al., 2009). The kinetics of MRD clearance, assessed by changes in MRD level between day 33 and 78, also demonstrated predictive ability; persistent low levels of MRD beyond Consolidation also portend an adverse outcome.

In the COG study P9900, MRD assessed by FCM in peripheral blood at day 8 and bone marrow at day 29 (end of Induction) in nearly 2000 patients predicted long term EFS. Informative end-Induction MRD results with a sensitivity of 0.01% performed in a reference laboratory were available for 92% of patients within 48 hrs of specimen receipt. End-Induction MRD level was highly prognostic with 5 year EFS of 88+/-1%, 59+/-5%, and 30+/-8% for MRD levels of 0.01-.1%, 0.1-1%, and >1% respectively. These results confirmed 0.01% as the optimal cutoff level for risk stratification in risk-adjusted treatment studies. End-Induction MRD was again found to be the most important prognostic variable in multivariate analysis in this study (Borowitz et al., 2008). Current risk stratification in COG ALL studies of low risk B cell precursor ALL includes both day 8 peripheral blood and day 29 bone marrow MRD and defines a group of patients with a predicted 5 year EFS exceeding 95% (Hunger et al., 2010).

Whereas the majority of ALL, particularly in children, is of B cell precursor origin, the importance of MRD as a prognostic indicator has also been evaluated in T cell ALL. In the AEIOP-BFM 2000 study, T cell patients were prospectively evaluated and observed to have delayed kinetics of MRD clearance when compared to B precursor ALL patients. Nonetheless, negative MRD levels at either/both days 33 and 78 were highly predictive of a favorable outcome. Only 16% of T cell ALL patients were MRD negative at day 33 and experienced a relapse rate of 7%; 32% of patients who were MRD positive at day 33 achieved negative MRD levels by day 78 and this group had a relapse rate of 9% (Willemse et al., 2002; Schrappe et al., 2007). Thus, although the different kinetics of MRD clearance in T cell ALL reflect the well established difference in biology and response to therapy, the value of MRD as a prognostic biomarker in the setting of controlled clinical trials appears established and may require special consideration with respect to selection and modification of the currently available and accepted techniques for assessing MRD levels.

Of particular significance are important prognostic observations in discrete subpopulations of pediatric patients with B cell precursor ALL who are at high risk for treatment failure. The prognostic impact of early MRD determination in the biologically unique group of infants < 1 year of age has been reported (van der Velden et al., 2009). Another rare, but distinct group of pediatric patients includes those with mature B cell ALL for whom early MRD levels also appear prognostically relevant (Mussolin et al., 2010). Risk of relapse in patients with the intrachromosomal amplification of chromosome 21 can be de differentiated based on early MRD determination (Attarbaschi et al., 2008). Early MRD determination also provides important prognostic information in children with Ph+ ALL treated prior to the incorporation of BCR/ABL1 specific tyrosine kinase inhibitors into conventional treatment regimens (Biondi et al., 2008; Conter et al., 2010).

The prognostic significance of early MRD in ALL is not limited to the pediatric experience. There is increasing evidence for the prognostic significance of early MRD levels in adults with ALL treated with intensive, multi-agent regimens similar to those utilized in the pediatric population especially those conducted by the German Multicenter ALL (GMALL) Group (Brugemann et al., 2006). The finding of MRD levels <0.01% within the first 3 weeks of therapy was associated with a 3 year relapse rate of 0% in contrast to a 94% relapse rate in patients with persistent MRD levels of 0.01% up to week 16 of therapy. The use of early MRD assessment in risk stratification of adult patients with ALL treated with modern multi-agent chemotherapy on

clinical trials of the GMALL study group and the Northern Italy Leukaemia Group (NILG) have resulted in marked improvement in long term outcomes for adult patients (Bruggemann at al., 2006; Bassan et al., 2009). In addition, results of the ALL 4-2002 study of the Polish Adult Leukemia Group demonstrated the independent prognostic value of end-Induction MRD assessed by multiparametric FCM (Holowiecki et al., 2008). This experience has resulted in the widely disseminated opinion that, as in children, MRD in adults with ALL is such an important prognostic variable that treatment decisions and risk stratifications in clinical trials should incorporate this factor (Bassan and Hoelzer, 2011).

Negative early MRD levels appear to have prognostic significance in the setting of first hematological relapse (Eckert et al., 2001; Paganin et al., 2008; Raetz et al., 2008) and in isolated extramedullary relapse (Hagedorn et al., 2007). In the COG AALL01P1 study, negative MRD status at the completion of the first course of Induction therapy was predictive of a superior 1 year EFS compared to patients who remained MRD positive (Raetz et al., 2008). In a subsequent study of ALL in first relapse, the addition of an investigational agent, epratuzumab to the same Induction chemotherapy block increased the incidence of MRD negativity and the correlation with long term outcome awaits evaluation (Raetz et al., 2011). However, conflicting observations have arisen from the UKALL R3 study comparing idarubicin to mitoxantrone in children with relapsed ALL where differences in subsequent relapse free survival were observed without corresponding differences in end-Induction MRD levels (V. Saha, personal communication).

Importantly, negative MRD levels prior to stem cell transplantation in both relapsed patients and in those transplanted in first remission are associated with superior relapse-free survival when compared to the outcomes of patients with persistent MRD positivity at time of transplant (Bader et al., 2002, 2009; Cario et al., 2008; Foster et al., 2011). Extending the predictive utility of post-Induction MRD to the treatment planning and decision-making incorporating specific transplantation strategies provides a rationale for future clinical investigations and confirms the role of MRD as a useful biomarker for in vivo response to therapy.

The evidence base to indicate that early MRD status is the strongest predictor of long term EFS in ALL is unequivocal. The magnitude of the importance of its current, critical role in risk stratification for treatment decisions has raised the consideration of its potential as a surrogate endpoint for clinical trials of investigational therapeutic interventions. The significant increase in sensitivity afforded by accepted techniques to assess MRD compared to standard techniques to define objective response and complete remission in this disease could influence future considerations and standards for response assessment and hasten the development timeline for new drugs and biologics for ALL. Adopting an important prognostic biomarker as a reliable response biomarker and surrogate for new agent efficacy requires both careful prospective evaluation in future controlled trials and standardization of sensitive techniques for which exacting performance standards have been established.

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